

WHAT IS CLAIMED IS:

1 1. A method for preparing a fluorescent dye-labeled biological agent, said
2 method comprising contacting an unlabeled biological agent with a fluorescent dye-fused
3 lactone derivative under conditions sufficient to covalently attach the fluorescent dye to said
4 biological agent and form a fluorescent dye-labeled biological agent.

1 2. A method for preparing a fluorescent dye-labeled phosphoramidite
2 reagent, said method comprising:

3 (a) contacting a fluorescent dye-fused lactone derivative with a linking group
4 component to form an intermediate fluorescent dye-labeled linking group; and

5 (b) contacting said intermediate fluorescent dye-labeled linking group with a
6 phosphoramidite moiety under conditions sufficient to covalently attach the phosphoramidite
7 moiety to said fluorescent dye-labeled linking group and form said fluorescent dye-labeled
8 phosphoramidite reagent.

1 3. A method in accordance with claim 2, wherein said fluorescent dye-
2 fused lactone derivative has a formula selected from the group consisting of I, II, III, IV, V,
3 VI, VII, VIII, and IX.

1 4. A method in accordance with claim 2, wherein fluorescent dye-fused
2 lactone derivative has a formula selected from the group consisting of Ia, Ib, IIa, IIb, IIIa,
3 IIb, IVa, IVb, V and VI.

1 5. A method in accordance with claim 2, wherein said linking group
2 component comprises two reactive functional groups selected from amino, hydroxy,
3 hydrazino and thiol.

1 6. A method in accordance with claim 2, wherein said linking group is
2 linear or cyclic or a combination thereof.

1 7. A method in accordance with claim 2, wherein said linking group
2 comprises a (C₂-C₂₀)alkylene or (C₂-C₂₀)heteroalkylene group.

1 8. A method in accordance with claim 2, wherein said linking group is
2 cyclic and comprises a five-membered heterocycle.

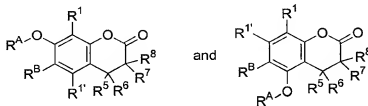
9. A method in accordance with claim 8, wherein said cyclic linking group is a prolinol linker.

10. A method in accordance with claim 1, wherein said biological agent is an oligonucleotide.

11. A method in accordance with claim 1, wherein said biological agent is an oligonucleotide having one or more modified bases.

12. A method in accordance with claim 1, wherein said fluorescent dye-fused lactone derivative is a member selected from the group consisting of coumarins, benzocoumarins, xanthenes, benzo[a]xanthenes, benzo[b]xanthenes, benzo[c]xanthenes, phenoxazines, benzo[a]phenoxazines, benzo[b]phenoxazines and benzo[c]phenoxazines.

13. A method in accordance with claim 1, wherein said fluorescent dye-fused lactone derivative has a formula selected from the group consisting of:



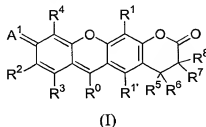
wherein

R¹ and R^{1'} are each members independently selected from the group consisting of H, halogen, cyano, CF₃, (C₁-C₈)alkyl, (C₁-C₈)alkylthio, (C₁-C₈)alkoxy, aryl and heteroaryl;

R⁵, R⁶, R⁷ and R⁸ are each independently selected from the group consisting of H, (C₁-C₈)alkyl, aryl, heteroaryl, aryl(C₁-C₄)alkyl and heteroaryl(C₁-C₄)alkyl; wherein the alkyl portions of any of R¹, R^{1'}, and R⁵ through R⁸ are optionally substituted with halogen, carboxy, sulfo, amino, mono- or dialkylamino, alkoxy, cyano, haloacetyl or hydroxy, and the alkyl portions of the substituents have from 1 to 6 carbon atoms; and the aryl or heteroaryl portions of any of R¹, R^{1'}, and R⁵ through R⁸ are optionally substituted with from one to four substituents selected from the group consisting of

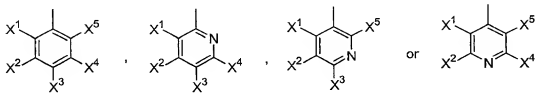
halogen, cyano, carboxy, sulfo, hydroxy, amino, mono- or di(C₁-C₆)alkylamino, (C₁-C₆)alkyl, (C₁-C₆)alkylthio and (C₁-C₆)alkoxy;
R^A and R^B are combined to form a substituted or unsubstituted fused ring system
having from 1 to 4 five- or six-membered rings; with the proviso that the
compound has an emission wavelength of from 400 nm to 1200 nm.

14. A method in accordance with claim 13, wherein said fluorescent dye-fused lactone derivative has the formula:



wherein

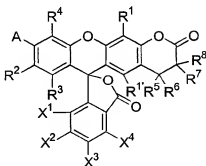
A¹ represents O or N-Z in which Z is H or (C₁-C₈)alkyl, or is optionally combined with R² or R⁴ to form a 5- or 6-membered ring or is combined with each of R² and R⁴ to form two fused 6-membered rings;
R¹, R², R³ and R⁴ are each independently selected from H, halogen, cyano, CF₃, (C₁-C₈)alkyl, (C₁-C₈)alkylthio, (C₁-C₈)alkoxy, aryl and heteroaryl;
R⁵, R⁶, R⁷ and R⁸ are each independently selected from H, (C₁-C₈)alkyl, aryl, heteroaryl, aryl(C₁-C₄)alkyl and heteroaryl(C₁-C₄)alkyl; wherein the alkyl portions of any of R¹ and R¹ through R⁸ are optionally substituted with halogen, carboxy, sulfo, amino, mono- or dialkylamino, alkoxy, cyano, haloacetyl or hydroxy, and the alkyl portions of the substituents have from 1 to 6 carbon atoms; and the aryl portions of any of R¹ and R¹ through R⁸ are optionally substituted with from one to four substituents selected from the group consisting of halogen, cyano, carboxy, sulfo, hydroxy, amino, mono- or di(C₁-C₆)alkylamino, (C₁-C₆)alkyl, (C₁-C₆)alkylthio and (C₁-C₆)alkoxy;
R⁰ is halogen, cyano, CF₃, (C₁-C₈)alkyl, (C₁-C₈)alkenyl, (C₁-C₈)alkynyl, substituted or unsubstituted heteroaryl or aryl having the formula:



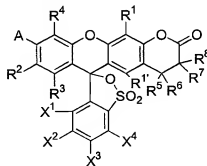
wherein X^1 , X^2 , X^3 , X^4 and X^5 are each independently selected from the group consisting of H, halogen, cyano, CF_3 , (C_1-C_8) alkyl, (C_1-C_8) alkoxy, (C_1-C_8) alkylthio, (C_1-C_8) alkenyl, (C_1-C_8) alkynyl, SO_3H and CO_2H , and optionally, any two adjacent X^1 through X^5 are combined to form an aromatic or heteroaromatic ring.

15. A method in accordance with claim 14, wherein R^1 is selected from the group consisting of H, halogen and phenyl; and each R^2 , R^3 and R^4 is independently selected from the group consisting of H and halogen.

16. A method in accordance with claim 14, wherein said fluorescent dye-fused lactone derivative has a formula selected from the group consisting of:



(Ia)



(Ib)

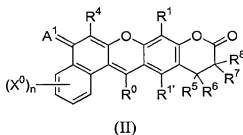
wherein

A is hydroxy, amino, protected hydroxy, or protected amino;
 R^1 , R^2 , R^3 and R^4 are each independently selected from H, halogen, cyano, CF_3 , (C_1-C_8) alkyl, (C_1-C_8) alkylthio and (C_1-C_8) alkoxy;
 R^5 , R^6 , R^7 and R^8 are each independently selected from H, (C_1-C_8) alkyl, aryl and aryl (C_1-C_4) alkyl; wherein the alkyl portions of any of R^1 and R^1 through R^8 are optionally substituted with halogen, carboxy, sulfo, amino, mono- or dialkylamino, alkoxy, cyano, haloacetyl or hydroxy, and the alkyl portions of the substituents have from 1 to 6 carbon atoms; and the aryl portions of any of R^5 through R^8 are optionally substituted with from one to four substituents selected from the group consisting of halogen, cyano, carboxy, sulfo, hydroxy, amino, mono- or di (C_1-C_6) alkylamino, (C_1-C_6) alkyl, (C_1-C_6) alkylthio and (C_1-C_6) alkoxy; and
 optionally, any two adjacent substituents X^1 through X^4 can be taken together to form a fused aromatic or heteroaromatic ring that is optionally further substituted

with from one to four substituents selected from halogen cyano, carboxy, sulfo, hydroxy, amino, mono- or di(C₁-C₆)alkylamino, (C₁-C₆)alkyl, (C₁-C₆)alkylthio and (C₁-C₆)alkoxy.

17. A method in accordance with claim 16, wherein said fluorescent dye-fused lactone derivative has formula Ia in which A is hydroxy or a protected hydroxy; R⁵ through R⁸ are each H; R¹, R², R³, R⁴ and R^{1'} are independently selected from the group consisting of H, halogen, cyano and CF₃.

18. A method in accordance with claim 13, wherein said fluorescent dye-fused lactone derivative has the formula:



wherein

A¹ is O or N-Z in which Z is H or (C₁-C₈)alkyl, or is optionally combined with R⁴ to form a 5- or 6-membered ring;

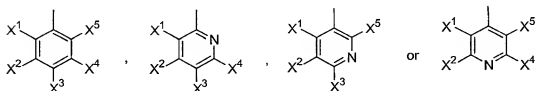
R^{1'}, R¹ and R⁴ are each independently selected from H, halogen, cyano, CF₃, (C₁-C₈)alkyl, (C₁-C₈)alkylthio, (C₁-C₈)alkoxy, aryl and heteroaryl;

R⁵, R⁶, R⁷ and R⁸ are each independently selected from H, (C₁-C₈)alkyl, aryl, heteroaryl, aryl(C₁-C₄)alkyl and heteroaryl(C₁-C₄)alkyl; wherein the alkyl portions of any of R^{1'} and R¹ through R⁸ are optionally substituted with halogen, carboxy, sulfo, amino, mono- or dialkylamino, alkoxy, cyano, haloacetyl or hydroxy, and the alkyl portions of the substituents have from 1 to 6 carbon atoms; and the aryl portions of any of R^{1'} and R¹ through R⁸ are optionally substituted with from one to four substituents selected from the group consisting of halogen, cyano, carboxy, sulfo, hydroxy, amino, mono- or di(C₁-C₆)alkylamino, (C₁-C₆)alkyl, (C₁-C₆)alkylthio and (C₁-C₆)alkoxy;

the subscript n is an integer of from 0 to 4;

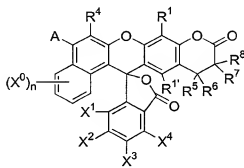
each X⁰ is a member independently selected from the group consisting of H, halogen, cyano, CF₃, (C₁-C₈)alkyl, (C₁-C₈)alkoxy, (C₁-C₈)alkylthio, (C₁-C₈)alkenyl, (C₁-C₈)alkynyl, aryl, heteroaryl, SO₃H and CO₂H;

R^0 is halogen, cyano, CF_3 , $(C_1-C_8)alkyl$, $(C_1-C_8)alkenyl$, $(C_1-C_8)alkynyl$, substituted or unsubstituted heteroaryl or aryl having the formula:



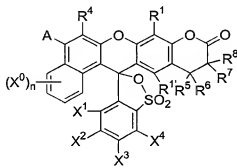
wherein X^1 , X^2 , X^3 , X^4 and X^5 are each independently selected from the group consisting of H, halogen, cyano, CF_3 , $(C_1-C_8)alkyl$, $(C_1-C_8)alkoxy$, $(C_1-C_8)alkylthio$, $(C_1-C_8)alkenyl$, $(C_1-C_8)alkynyl$, SO_3H and CO_2H , and optionally, any two adjacent X^1 through X^5 are combined to form an aromatic or heteroaromatic ring.

19. A method in accordance with claim 18, wherein said has a formula selected from the group consisting of:



IIa

and



IIc

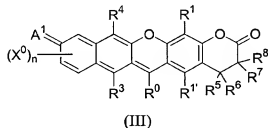
wherein

A is hydroxy, amino, protected hydroxy, or protected amino;
 $R^{1'}$, R^1 and R^4 are each independently selected from H, halogen, cyano, CF_3 , $(C_1-C_8)alkyl$, $(C_1-C_8)alkylthio$ and $(C_1-C_8)alkoxy$;
 R^5 , R^6 , R^7 and R^8 are each independently selected from H, $(C_1-C_8)alkyl$, aryl and aryl $(C_1-C_4)alkyl$; wherein the alkyl portions of any of $R^{1'}$ and R^1 through R^8 are optionally substituted with halogen, carboxy, sulfo, amino, mono- or dialkylamino, alkoxy, cyano, haloacetyl or hydroxy, and the alkyl portions of the substituents have from 1 to 6 carbon atoms; and the aryl portions of any of R^5 through R^8 are optionally substituted with from one to four substituents selected from the group consisting of halogen, cyano, carboxy, sulfo, hydroxy, amino, mono- or di $(C_1-C_6)alkylamino$, $(C_1-C_6)alkyl$, $(C_1-C_6)alkylthio$ and $(C_1-$

C₆alkoxy; and optionally, any two adjacent substituents X¹ through X⁴ can be taken together to form a fused aromatic or heteroaromatic ring that is optionally further substituted with from one to four substituents selected from halogen, cyano, carboxy, sulfo, hydroxy, amino, mono- or di(C₁-C₆)alkylamino, (C₁-C₆)alkyl, (C₁-C₆)alkylthio and (C₁-C₆)alkoxy.

20. A method in accordance with claim 19, wherein said fluorescent dye-fused lactone derivative has formula IIa in which A is hydroxy or a protected hydroxy; R⁵ through R⁸ are each H; R¹, R⁴ and R^{1'} are independently selected from the group consisting of H, halogen, cyano and CF₃.

21. A method in accordance with claim 12, wherein said benzo[b]xanthene has the formula:

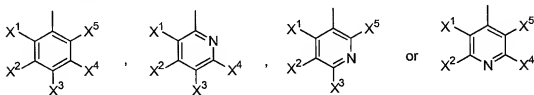


wherein

A¹ is O or N-Z in which Z is H or (C₁-C₈)alkyl, or is optionally combined with an adjacent X⁰ to form a 5- or 6-membered ring or is combined with two adjacent X⁰ groups to form two fused 6-membered rings;
 R^{1'}, R¹, R³ and R⁴ are each independently selected from H, halogen, cyano, CF₃, (C₁-C₈)alkyl, (C₁-C₈)alkylthio, (C₁-C₈)alkoxy, aryl and heteroaryl;
 R⁵, R⁶, R⁷ and R⁸ are each independently selected from H, (C₁-C₈)alkyl, aryl, heteroaryl, aryl(C₁-C₄)alkyl and heteroaryl(C₁-C₄)alkyl; wherein the alkyl portions of any of R^{1'} and R¹ through R⁸ are optionally substituted with halogen, carboxy, sulfo, amino, mono- or dialkylamino, alkoxy, cyano, haloacetyl or hydroxy, and the alkyl portions of the substituents have from 1 to 6 carbon atoms; and the aryl portions of any of R^{1'} and R¹ through R⁸ are optionally substituted with from one to four substituents selected from the group consisting of halogen, cyano, carboxy, sulfo, hydroxy, amino, mono- or di(C₁-C₆)alkylamino, (C₁-C₆)alkyl, (C₁-C₆)alkylthio and (C₁-C₆)alkoxy;
 the subscript n is an integer of from 0 to 4;

each X^0 is a member independently selected from the group consisting of H, halogen, cyano, CF_3 , $(C_1-C_8)alkyl$, $(C_1-C_8)alkoxy$, $(C_1-C_8)alkylthio$, $(C_1-C_8)alkenyl$, $(C_1-C_8)alkynyl$, aryl, heteroaryl, SO_3H and CO_2H ;

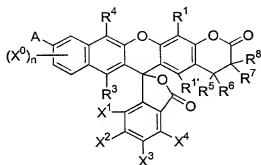
R^0 is a member selected from the group consisting of halogen, cyano, CF_3 , $(C_1-C_8)alkyl$, $(C_1-C_8)alkenyl$, $(C_1-C_8)alkynyl$, and substituted or unsubstituted heteroaryl or aryl having the formula:



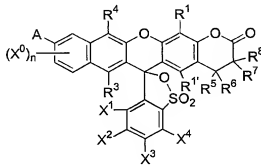
wherein X^1 , X^2 , X^3 , X^4 and X^5 are each independently selected from the group consisting of H, halogen, cyano, CF_3 , $(C_1-C_8)alkyl$, $(C_1-C_8)alkoxy$, $(C_1-C_8)alkylthio$, $(C_1-C_8)alkenyl$, $(C_1-C_8)alkynyl$, SO_3H and CO_2H , and optionally, any two adjacent X^1 through X^5 are combined to form an aromatic or heteroaromatic ring.

22. A method in accordance with claim 21, wherein R^1 is halogen; and each X^1 through X^4 is independently selected from the group consisting of H, F and Cl.

23. A method in accordance with claim 21, wherein said benzo[b]xanthene has a formula selected from the group consisting of:



(IIIa)



(IIIc)

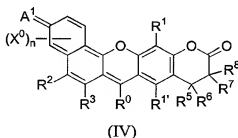
wherein

A is hydroxy, amino, protected hydroxy, or protected amino;
the subscript n is an integer of from 0 to 3;
each X^0 is a member independently selected from the group consisting of H, halogen, cyano, CF_3 , $(C_1-C_8)alkyl$, $(C_1-C_8)alkoxy$, $(C_1-C_8)alkylthio$, $(C_1-C_8)alkenyl$, $(C_1-C_8)alkynyl$, aryl, heteroaryl, SO_3H and CO_2H ;

R^{1'}, R¹, R³ and R⁴ are each independently selected from H, halogen, cyano, CF₃, (C₁-C₈)alkyl, (C₁-C₈)alkylthio and (C₁-C₈)alkoxy;
R⁵, R⁶, R⁷ and R⁸ are each independently selected from H, (C₁-C₈)alkyl, aryl and aryl(C₁-C₄)alkyl; wherein the alkyl portions of any of R^{1'} and R¹ through R⁸ are optionally substituted with halogen, carboxy, sulfo, amino, mono- or dialkylamino, alkoxy, cyano, haloacetyl or hydroxy, and the alkyl portions of the substituents have from 1 to 6 carbon atoms; and the aryl portions of any of R⁵ through R⁸ are optionally substituted with from one to four substituents selected from the group consisting of halogen, cyano, carboxy, sulfo, hydroxy, amino, mono- or di(C₁-C₆)alkylamino, (C₁-C₆)alkyl, (C₁-C₆)alkylthio and (C₁-C₆)alkoxy; and optionally, any two adjacent substituents X¹ through X⁴ can be taken together to form a fused aromatic or heteroaromatic ring that is optionally further substituted with from one to four substituents selected from halogen cyano, carboxy, sulfo, hydroxy, amino, mono- or di(C₁-C₆)alkylamino, (C₁-C₆)alkyl, (C₁-C₆)alkylthio and (C₁-C₆)alkoxy.

24. A method in accordance with claim 23, wherein said fluorescent dye-fused lactone derivative has formula IIIa in which A is hydroxy or a protected hydroxy; R⁵ through R⁸ are each H; R¹, R³, R⁴ and R^{1'} are independently selected from the group consisting of H, halogen, cyano and CF₃.

25. A method in accordance with claim 12, wherein said benzo[c]xanthene has the formula:

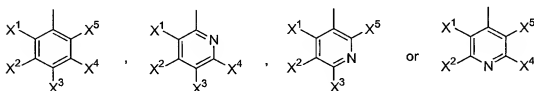


wherein

A¹ is O or N-Z in which Z is H or (C₁-C₈)alkyl, or is optionally combined with an adjacent X⁰ to form a 5- or 6-membered ring or is combined with two adjacent X⁰ groups to form two fused 6-membered rings;

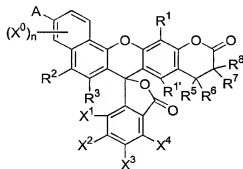
R^{1'}, R¹ and R⁴ are each independently selected from H, halogen, cyano, CF₃, (C₁-C₈)alkyl, (C₁-C₈)alkylthio, (C₁-C₈)alkoxy, aryl and heteroaryl;

R^5 , R^6 , R^7 and R^8 are each independently selected from H, (C₁-C₈)alkyl, aryl, heteroaryl, aryl(C₁-C₄)alkyl and heteroaryl(C₁-C₄)alkyl; wherein the alkyl portions of any of $R^{1'}$ and R^1 through R^8 are optionally substituted with halogen, carboxy, sulfo, amino, mono- or dialkylamino, alkoxy, cyano, haloacetyl or hydroxy, and the alkyl portions of the substituents have from 1 to 6 carbon atoms; and the aryl portions of any of $R^{1'}$ and R^1 through R^8 are optionally substituted with from one to four substituents selected from the group consisting of halogen, cyano, carboxy, sulfo, hydroxy, amino, mono- or di(C₁-C₆)alkylamino, (C₁-C₆)alkyl, (C₁-C₆)alkylthio and (C₁-C₆)alkoxy; the subscript n is an integer of from 0 to 3; each X^0 is a member independently selected from the group consisting of H, halogen, cyano, CF₃, (C₁-C₈)alkyl, (C₁-C₈)alkoxy, (C₁-C₈)alkylthio, (C₁-C₈)alkenyl, (C₁-C₈)alkynyl, aryl, heteroaryl, SO₃H and CO₂H; R^0 is halogen, cyano, CF₃, (C₁-C₈)alkyl, (C₁-C₈)alkenyl, (C₁-C₈)alkynyl, substituted or unsubstituted heteroaryl or aryl having the formula:

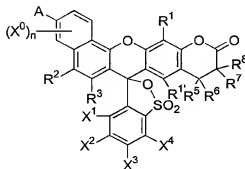


wherein X^1 , X^2 , X^3 , X^4 and X^5 are each independently selected from the group consisting of H, halogen, cyano, CF₃, (C₁-C₈)alkyl, (C₁-C₈)alkoxy, (C₁-C₈)alkylthio, (C₁-C₈)alkenyl, (C₁-C₈)alkynyl, SO₃H and CO₂H, and optionally, any two adjacent X^1 through X^5 are combined to form an aromatic or heteroaromatic ring.

26. A method in accordance with claim 25, wherein said benzo[c]xanthene has a formula selected from the group consisting of:



(IVa)



(IVc)

wherein

A is hydroxy, amino, protected hydroxy, or protected amino;

the subscript n is an integer of from 0 to 3;

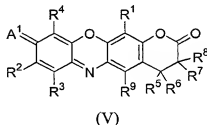
each X^0 is a member independently selected from the group consisting of H, halogen, cyano, CF_3 , $(C_1-C_8)alkyl$, $(C_1-C_8)alkoxy$, $(C_1-C_8)alkylthio$, $(C_1-C_8)alkenyl$, $(C_1-C_8)alkynyl$, aryl, heteroaryl, SO_3H and CO_2H ;

$R^{1'}$, R^1 , R^3 and R^4 are each independently selected from H, halogen, cyano, CF_3 , $(C_1-C_8)alkyl$, $(C_1-C_8)alkylthio$ and $(C_1-C_8)alkoxy$;

R^5 , R^6 , R^7 and R^8 are each independently selected from H, $(C_1-C_8)alkyl$, aryl and aryl $(C_1-C_4)alkyl$; wherein the alkyl portions of any of $R^{1'}$ and R^1 through R^8 are optionally substituted with halogen, carboxy, sulfo, amino, mono- or dialkylamino, alkoxy, cyano, haloacetyl or hydroxy, and the alkyl portions of the substituents have from 1 to 6 carbon atoms; and the aryl portions of any of R^5 through R^8 are optionally substituted with from one to four substituents selected from the group consisting of halogen, cyano, carboxy, sulfo, hydroxy, amino, mono- or di $(C_1-C_6)alkylamino$, $(C_1-C_6)alkyl$, $(C_1-C_6)alkylthio$ and $(C_1-C_6)alkoxy$; and optionally, any two adjacent substituents X^1 through X^4 can be taken together to form a fused aromatic or heteroaromatic ring that is optionally further substituted with from one to four substituents selected from halogen cyano, carboxy, sulfo, hydroxy, amino, mono- or di $(C_1-C_6)alkylamino$, $(C_1-C_6)alkyl$, $(C_1-C_6)alkylthio$ and $(C_1-C_6)alkoxy$.

27. A method in accordance with claim 26, wherein said fluorescent dye-fused lactone derivative has formula IIa in which A is hydroxy or a protected hydroxy; R^5 through R^8 are each H; R^1 , R^2 , R^3 and $R^{1'}$ are independently selected from the group consisting of H, halogen, cyano and CF_3 .

28. A method in accordance with claim 12, wherein said phenoxazine has the formula:



wherein

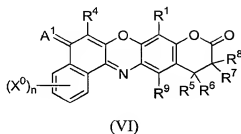
A¹ is O or N-Z in which Z is H or (C₁-C₈)alkyl, or is optionally combined with R² or R⁴ to form a 5- or 6-membered ring or is combined with each of R² and R⁴ to form two fused 6-membered rings;

R¹, R², R³, R⁴ and R⁹ are each independently selected from the group consisting of H, halogen, cyano, CF₃, (C₁-C₈)alkyl, (C₁-C₈)alkylthio, (C₁-C₈)alkoxy, aryl and heteroaryl;

R⁵, R⁶, R⁷ and R⁸ are each independently selected from H, (C₁-C₈)alkyl, aryl, heteroaryl, aryl(C₁-C₄)alkyl and heteroaryl(C₁-C₄)alkyl; wherein the alkyl portions of any of R¹ through R⁹ are optionally substituted with halogen, carboxy, sulfo, amino, mono- or dialkylamino, alkoxy, cyano, haloacetyl or hydroxy, and the alkyl portions of the substituents have from 1 to 6 carbon atoms; and the aryl portions of any of R¹ through R⁹ are optionally substituted with from one to four substituents selected from the group consisting of halogen, cyano, carboxy, sulfo, hydroxy, amino, mono- or di(C₁-C₆)alkylamino, (C₁-C₆)alkyl, (C₁-C₆)alkylthio and (C₁-C₆)alkoxy.

29. A method in accordance with claim 28, wherein said fluorescent dye-fused lactone derivative has formula V in which A¹ is O; R⁵ through R⁸ are each H; R¹ through R⁴ and R⁹ are independently selected from the group consisting of H, halogen, cyano and CF₃.

30. A method in accordance with claim 12, wherein said benzo[a]phenoxazines have the formula:



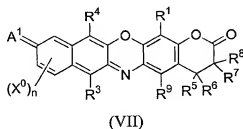
wherein

A¹ is O or N-Z in which Z is H or (C₁-C₈)alkyl, or is optionally combined with R⁴ to form a 5- or 6-membered ring;

R^1 , R^4 and R^9 are each independently selected from the group consisting of H,
 halogen, cyano, CF_3 , (C_1-C_8) alkyl, (C_1-C_8) alkylthio, (C_1-C_8) alkoxy, aryl and
 heteroaryl;
 R^5 , R^6 , R^7 and R^8 are each independently selected from the group consisting of H,
 (C_1-C_8) alkyl, aryl, heteroaryl, aryl (C_1-C_4) alkyl and heteroaryl (C_1-C_4) alkyl;
 wherein the alkyl portions of any of R^1 through R^9 are optionally substituted with
 halogen, carboxy, sulfo, amino, mono- or dialkylamino, alkoxy, cyano,
 haloacetyl or hydroxy, and the alkyl portions of the substituents have from 1 to
 6 carbon atoms; and the aryl portions of any of R^1 through R^9 are optionally
 substituted with from one to four substituents selected from the group
 consisting of halogen, cyano, carboxy, sulfo, hydroxy, amino, mono- or di $(C_1-$
 $C_6)$ alkylamino, (C_1-C_6) alkyl, (C_1-C_6) alkylthio and (C_1-C_6) alkoxy;
 the subscript n is an integer of from 0 to 4; and
 each X^0 is independently selected from the group consisting of H, halogen, cyano,
 CF_3 , (C_1-C_8) alkyl, (C_1-C_8) alkoxy, (C_1-C_8) alkylthio, (C_2-C_8) alkenyl, $(C_2-$
 $C_8)$ alkynyl, aryl, heteroaryl, SO_3H and CO_2H .

31. A method in accordance with claim 30, wherein said fluorescent dye-
 fused lactone derivative has formula VI in which A^1 is O; R^5 through R^8 are each H; R^1 , R^4
 and R^9 are independently selected from the group consisting of H, halogen, cyano and CF_3 .

32. A method in accordance with claim 12, wherein said
 benzo[b]phenoxazines have the formula:



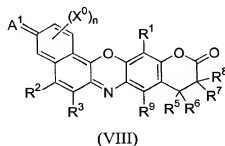
wherein

A^1 is O or N-Z in which Z is H or (C_1-C_8) alkyl, or is optionally combined with an
 adjacent X^0 to form a 5- or 6-membered ring or is combined with two adjacent
 X^0 groups to form two fused 6-membered rings;
 R^1 , R^3 , R^4 and R^9 are each independently selected from H, halogen, cyano, CF_3 , $(C_1-$
 $C_8)$ alkyl, (C_1-C_8) alkylthio, (C_1-C_8) alkoxy, aryl and heteroaryl;

R^5 , R^6 , R^7 and R^8 are each independently selected from the group consisting of H,
 (C_1-C_8) alkyl, aryl, heteroaryl, aryl(C_1-C_4)alkyl and heteroaryl(C_1-C_4)alkyl;
 wherein the alkyl portions of any of R^1 or R^3 through R^9 are optionally substituted
 with halogen, carboxy, sulfo, amino, mono- or dialkylamino, alkoxy,
 cyano, haloacetyl or hydroxy, and the alkyl portions of the substituents
 have from 1 to 6 carbon atoms; and the aryl or heteroaryl portions of any
 of R^1 and R^3 through R^9 are optionally substituted with from one to four
 substituents selected from the group consisting of halogen, cyano, carboxy,
 sulfo, hydroxy, amino, mono- or di(C_1-C_6)alkylamino, (C_1-C_6)alkyl, (C_1 -
 C_6)alkylthio and (C_1-C_6)alkoxy;
 each X^0 is independently selected from the group consisting of H, halogen, cyano,
 CF_3 , (C_1-C_8)alkyl, (C_1-C_8)alkoxy, (C_1-C_8)alkylthio, (C_1-C_8)alkenyl, (C_1 -
 C_8)alkynyl, aryl, heteroaryl, SO_3H and CO_2H . Additionally, the alkyl portions
 of any X^0 can be further substituted with halogen, carboxy, sulfo, amino,
 mono- or dialkylamino, alkoxy, cyano, haloacetyl or hydroxy, and the alkyl
 portions of the substituents have from 1 to 6 carbon atoms; and
 the subscript n is an integer of from 0 to 3.

33. A method in accordance with claim 32, wherein said fluorescent dye-
 fused lactone derivative has formula VII in which A^1 is O; R^5 through R^8 are each H; R^1 , R^3 ,
 R^4 and R^9 are independently selected from the group consisting of H, halogen, cyano and
 CF_3 .

34. A method in accordance with claim 12, wherein said
 benzo[c]phenoxazines have the formula:



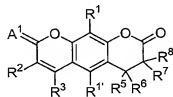
wherein

A^1 is O or N-Z in which Z is H or (C_1-C_8)alkyl, or is optionally combined with an
 adjacent X^0 to form a 5- or 6-membered ring or is combined with two adjacent
 X^0 groups to form two fused 6-membered rings;

R^1 , R^2 , R^3 and R^9 are each independently selected from H, halogen, cyano, CF_3 , $(C_1-C_8)alkyl$, $(C_1-C_8)alkylthio$, $(C_1-C_8)alkoxy$, aryl and heteroaryl;
 R^5 , R^6 , R^7 and R^8 are each independently selected from the group consisting of H, $(C_1-C_8)alkyl$, aryl, heteroaryl, aryl $(C_1-C_4)alkyl$ and heteroaryl $(C_1-C_4)alkyl$;
 wherein the alkyl portions of any of R^1 , R^2 , R^3 or R^5 through R^9 are optionally substituted with halogen, carboxy, sulfo, amino, mono- or dialkylamino, alkoxy, cyano, haloacetyl or hydroxy, and the alkyl portions of the substituents have from 1 to 6 carbon atoms; and the aryl or heteroaryl portions of any of R^1 , R^2 , R^3 or R^5 through R^9 are optionally substituted with from one to four substituents selected from the group consisting of halogen, cyano, carboxy, sulfo, hydroxy, amino, mono- or di $(C_1-C_6)alkylamino$, $(C_1-C_6)alkyl$, $(C_1-C_6)alkylthio$ and $(C_1-C_6)alkoxy$;
 each X^0 is independently selected from the group consisting of H, halogen, cyano, CF_3 , $(C_1-C_8)alkyl$, $(C_1-C_8)alkoxy$, $(C_1-C_8)alkylthio$, $(C_1-C_8)alkenyl$, $(C_1-C_8)alkynyl$, aryl, heteroaryl, SO_3H and CO_2H , wherein the alkyl or aryl portions of any X^0 can be further substituted with halogen, carboxy, sulfo, amino, mono- or dialkylamino, alkoxy, cyano, haloacetyl or hydroxy, and the alkyl portions of the substituents have from 1 to 6 carbon atoms; and
 the subscript n is an integer of from 0 to 3.

35. A method in accordance with claim 34, wherein said fluorescent dye-fused lactone derivative has formula VIII in which A^1 is O; R^5 through R^8 are each H; R^1 , R^2 , R^3 and R^9 are independently selected from the group consisting of H, halogen, cyano and CF_3 .

36. A method in accordance with claim 13; wherein said substituted coumarin has the formula:



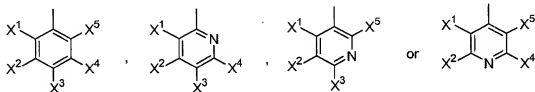
(IX)

wherein

R^1, R^1', R^2 and R^3 are each independently selected from the group consisting of H, halogen, cyano, CF_3 , (C_1-C_8) alkyl, (C_1-C_8) alkylthio, (C_1-C_8) alkoxy, aryl and heteroaryl;
 R^5, R^6, R^7 and R^8 are each independently selected from the group consisting of, (C_1-C_8) alkyl, aryl, heteroaryl, aryl (C_1-C_4) alkyl and heteroaryl (C_1-C_4) alkyl;
 wherein the alkyl portions of any of R^1' or R^1 through R^8 are optionally substituted with halogen, carboxy, sulfo, amino, mono- or dialkylamino, alkoxy, cyano, haloacetyl or hydroxy, and the alkyl portions of the substituents have from 1 to 6 carbon atoms; and the aryl or heteroaryl portions of any of R^1' and R^1 through R^8 are optionally substituted with from one to four substituents selected from the group consisting of halogen, cyano, carboxy, sulfo, hydroxy, amino, mono- or di (C_1-C_6) alkylamino, (C_1-C_6) alkyl, (C_1-C_6) alkylthio and (C_1-C_6) alkoxy, or optionally, R^2 and R^3 are taken together to form a fused aromatic ring;
 A^1 represents O or N-Z, in which Z is H or (C_1-C_8) alkyl.

37. A method in accordance with claim 36, wherein R^1 is H or halogen; R^3 is (C_1-C_4) alkyl; and R^1' and R^2 are each hydrogen.

38. A method in accordance with claim 36, wherein R^2 and R^3 are independently selected from halogen, cyano, CF_3 , (C_1-C_8) alkyl, and aryl or heteroaryl having the formula:

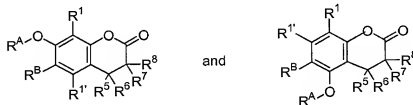


wherein

X^1, X^2, X^3, X^4 and X^5 are each independently selected from the group consisting of H, halogen, cyano, CF_3 , (C_1-C_8) alkyl, (C_1-C_8) alkoxy, (C_1-C_8) alkylthio, (C_1-C_8) alkenyl, (C_1-C_8) alkynyl, SO_3H and CO_2H , wherein the alkyl portions of any of X^1 through X^5 can be further substituted with halogen, carboxy, sulfo, amino, mono- or dialkylamino, alkoxy, cyano, haloacetyl or hydroxy, and the alkyl portions of the substituents have from 1 to 6 carbon atoms, and optionally, any two adjacent substituents X^1 through X^5 can be taken together to form a fused aromatic ring that is optionally further substituted with from

one to four substituents selected from halogen cyano, carboxy, sulfo, hydroxy, amino, mono- or di(C₁-C₆)alkylamino, (C₁-C₆)alkyl, (C₁-C₆)alkylthio and (C₁-C₆)alkoxy.

39. A fused lactone dye having a formula selected from:



wherein

R¹ and R^{1'} are each members independently selected from the group consisting of H, halogen, cyano, CF₃, (C₁-C₈)alkyl, (C₁-C₈)alkylthio, (C₁-C₈)alkoxy, aryl and heteroaryl;

R⁵, R⁶, R⁷ and R⁸ are each independently selected from the group consisting of H, (C₁-C₈)alkyl, aryl, heteroaryl, aryl(C₁-C₄)alkyl and heteroaryl(C₁-C₄)alkyl;

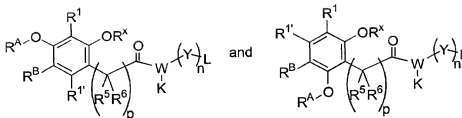
wherein the alkyl portions of any of R¹, R^{1'}, and R⁵ through R⁸ are optionally substituted with halogen, carboxy, sulfo, amino, mono- or dialkylamino, alkoxy, cyano, haloacetyl or hydroxy, and the alkyl portions of the substituents have from 1 to 6 carbon atoms; and the aryl or heteroaryl portions of any of R¹, R^{1'}, and R⁵ through R⁸ are optionally substituted with from one to four substituents selected from the group consisting of halogen, cyano, carboxy, sulfo, hydroxy, amino, mono- or di(C₁-C₆)alkylamino, (C₁-C₆)alkyl, (C₁-C₆)alkylthio and (C₁-C₆)alkoxy;

R^A and R^B are combined to form a substituted or unsubstituted fused ring system having from 1 to 4 five- or six-membered rings; with the proviso that the compound has an emission wavelength of from 400 nm to 1200 nm.

40. A fused-lactone dye of claim 39, having a formula selected from the group consisting of formula I, II, III, IV, V, VI, VII, VIII and IX.

41. A fused-lactone dye of claim 39, having a formula selected from the group consisting of formula Ia, Ib, IIa, IIb, IIIa, IIIb, IVa, IVb, V and VI.

42. A dye reagent having a formula selected from:



wherein

R^1 and $R^{1'}$ are each members independently selected from the group consisting of H, halogen, cyano, CF_3 , (C_1-C_8) alkyl, (C_1-C_8) alkylthio, (C_1-C_8) alkoxy, aryl and heteroaryl;

each R^5 and R^6 is independently selected from the group consisting of H, (C_1-C_8) alkyl, aryl, heteroaryl, aryl (C_1-C_4) alkyl and heteroaryl (C_1-C_4) alkyl;

wherein the alkyl portions of any of R^1 , $R^{1'}$, R^5 and R^6 are optionally substituted

with halogen, carboxy, sulfo, amino, mono- or dialkylamino, alkoxy, cyano, haloacetyl or hydroxy, and the alkyl portions of the substituents have from 1 to 6 carbon atoms; and the aryl or heteroaryl portions of any of R^1 , $R^{1'}$, R^5 and R^6 are optionally substituted with from one to four substituents selected from the group consisting of halogen, cyano, carboxy, sulfo, hydroxy, amino, mono- or di (C_1-C_6) alkylamino, (C_1-C_6) alkyl, (C_1-C_6) alkylthio and (C_1-C_6) alkoxy;

R^A and R^B are combined to form a substituted or unsubstituted fused ring system having from 1 to 4 five- or six-membered rings;

R^x is selected from the group consisting of H and hydroxy protecting groups;

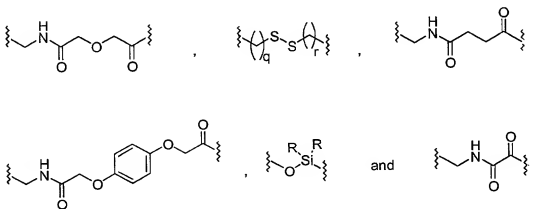
the subscript p is an integer of from 1 to 3;

W is a di-, tri- or tetravalent linker which is acyclic, cyclic, aromatic or a combination thereof, having from 4 to 50 atoms selected from the group consisting of C, N, O, P and S and exclusive of hydrogen atoms that fill available valences, and further having a nitrogen atom directly connected to the adjacent carbonyl group;

K is selected from the group consisting of a lone pair of electrons, H, OH, SH, NH, (C_1-C_8) alkyl, aryl, an amino protecting group and a hydroxy protecting group;

the subscript n is 0 or 1; and when n is 1, Y is a cleavable linking group and L is a solid support; and when n is 0, L is a phosphoramidite or reactive functional group the subscript p is an integer of from 1 to 3; with the proviso that the reagent has an emission wavelength of from 400 to 1200 nm.

43. A dye reagent in accordance with claim 42, wherein n is 1, and Y is selected from the group consisting of:

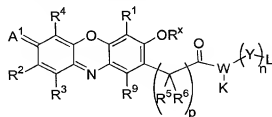


wherein the subscripts q and r are independently integers of from 1 to 15; and each R is independently (C₁-C₈)alkyl or (C₁-C₈)alkoxy.

44. A dye reagent in accordance with claim 42, wherein said reagent has an emission wavelength of from 400 nm to about 850nm.

45. A dye reagent in accordance with claim 42, wherein R^A, R^B and the ring to which each is attached forms a dye selected from the group consisting of a fluorescein, a benzocoumarin, a xanthene, a benzo[a]xanthene, a benzo[b]xanthene, a benzo[c]xanthene, a phenoxazine, a benzo[a]phenoxazine, a benzo[b]phenoxazine and a benzo[c]phenoxazine.

46. A dye reagent in accordance with claim 45, having the formula:



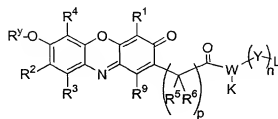
(XIIa)

wherein

A¹ is O or N-Z in which Z is H or (C₁-C₈)alkyl;

R^1, R^2, R^3, R^4 and R^9 are each independently selected from the group consisting of H, halogen, cyano, CF_3 , (C_1-C_8) alkyl, (C_1-C_8) alkylthio, (C_1-C_8) alkoxy, aryl and heteroaryl;
each R^5 and R^6 is independently selected from the group consisting of H, (C_1-C_8) alkyl, aryl, heteroaryl, aryl (C_1-C_4) alkyl and heteroaryl (C_1-C_4) alkyl;
wherein the alkyl portions of any of R^1 through R^9 are optionally substituted with halogen, carboxy, sulfo, amino, mono- or dialkylamino, alkoxy, cyano, haloacetyl or hydroxy, and the alkyl portions of the substituents have from 1 to 6 carbon atoms; and the aryl portions of any of R^1 through R^9 are optionally substituted with from one to four substituents selected from the group consisting of halogen, cyano, carboxy, sulfo, hydroxy, amino, mono- or di (C_1-C_6) alkylamino, (C_1-C_6) alkyl, (C_1-C_6) alkylthio and (C_1-C_6) alkoxy;
optionally, R^2 taken together with R^3 form a fused aromatic ring that is optionally substituted with from one to four substituents selected from halogen, cyano, carboxy, sulfo, hydroxy, amino, mono- or di (C_1-C_6) alkylamino, (C_1-C_6) alkyl, (C_1-C_6) alkylthio and (C_1-C_6) alkoxy;
and tautomeric forms thereof.

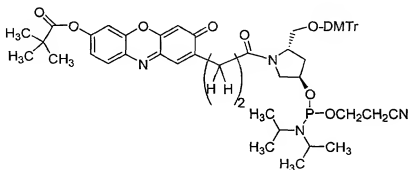
47. A dye reagent in accordance with claim 46, having the formula:



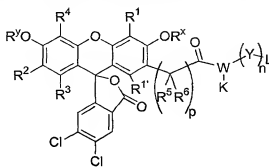
(XIIc)

wherein R^y is a protecting group.

48. A dye reagent of claim 47, having the formula:



49. A 5,6-dichlorofluorescein dye reagent having the formula:



wherein

R^x and R^y are H or independently selected protecting groups;

R^1 , R^2 , R^3 and R^4 are each independently selected from the group consisting of H, halogen, cyano, CF_3 , (C_1-C_8) alkyl, (C_1-C_8) alkylthio, (C_1-C_8) alkoxy, aryl and heteroaryl;

each R^5 and R^6 is independently selected from the group consisting of H, (C_1-C_8) alkyl, aryl, heteroaryl, aryl (C_1-C_4) alkyl and heteroaryl (C_1-C_4) alkyl;

wherein the alkyl portions of any of R^1 and R^1 through R^6 are optionally substituted with halogen, carboxy, sulfo, amino, mono- or dialkylamino, alkoxy, cyano, haloacetyl or hydroxy, and the alkyl portions of the substituents have from 1 to 6 carbon atoms; and the aryl portions of any of R^1 and R^1 through R^6 are optionally substituted with from one to four substituents selected from the group consisting of halogen, cyano, carboxy, sulfo, hydroxy, amino, mono- or di (C_1-C_6) alkylamino, (C_1-C_6) alkyl, (C_1-C_6) alkylthio and (C_1-C_6) alkoxy; optionally, R^2 taken together with R^3 form a fused aromatic or heteroaromatic ring that is optionally substituted with from one to four substituents selected from halogen cyano, carboxy, sulfo, hydroxy, amino, mono- or di (C_1-C_6) alkylamino, (C_1-C_6) alkyl, (C_1-C_6) alkylthio and (C_1-C_6) alkoxy;

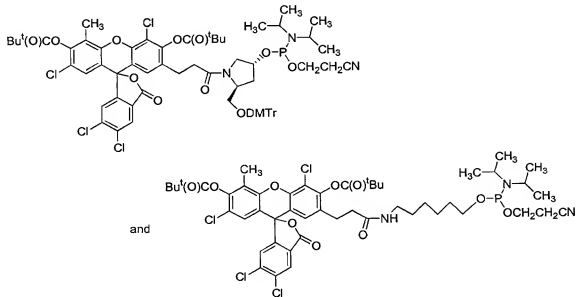
the subscript p is an integer of from 1 to 3;

W is a di-, tri- or tetra-valent linker which is acyclic, cyclic, aromatic or a combination thereof, having from 4 to 50 atoms selected from the group consisting of C, N, O, P and S and exclusive of hydrogen atoms that fill available valences, and further having a nitrogen atom directly connected to the adjacent carbonyl group;

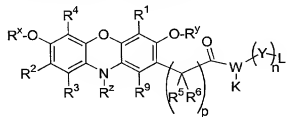
K is selected from the group consisting of a lone pair of electrons, H, OH, SH, NH, (C₁-C₈)alkyl, aryl, an amino protecting group and a hydroxy protecting group;
the subscript n is 0 or 1; and when n is 1, Y is a cleavable linking group and L is a solid support; and when n is 0, L is a phosphoramidite or reactive functional group the subscript p is an integer of from 1 to 3; and tautomeric forms thereof.

50. A 5,6-dichlorofluorescein dye reagent in accordance with claim 49, wherein R¹ is Cl; R⁵ and R⁶ are H; and the subscript p is 2.

51. A 5,6-dichlorofluorescein dye reagent in accordance with claim 50, having a formula selected from:



52. A leuco-phenoxazine dye reagent having the formula:

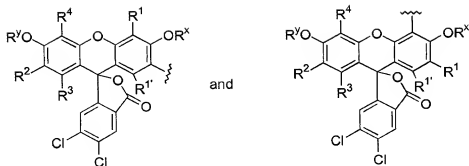


wherein

each of R^x, R^y and R^z are protecting groups;

R^1, R^2, R^3, R^4 and R^9 are each independently selected from the group consisting of H,
 halogen, cyano, CF_3 , (C_1-C_8) alkyl, (C_1-C_8) alkylthio, (C_1-C_8) alkoxy, aryl and
 heteroaryl;
 each R^5 and R^6 is independently selected from the group consisting of H, $(C_1-$
 $C_8)$ alkyl, aryl, heteroaryl, aryl (C_1-C_4) alkyl and heteroaryl (C_1-C_4) alkyl;
 wherein the alkyl portions of any of R^1 through R^9 are optionally substituted with
 halogen, carboxy, sulfo, amino, mono- or dialkylamino, alkoxy, cyano,
 haloacetyl or hydroxy, and the alkyl portions of the substituents have from 1 to
 6 carbon atoms; and the aryl portions of any of R^1 through R^9 are optionally
 substituted with from one to four substituents selected from the group
 consisting of halogen, cyano, carboxy, sulfo, hydroxy, amino, mono- or di- $(C_1-$
 $C_6)$ alkylamino, (C_1-C_6) alkyl, (C_1-C_6) alkylthio and (C_1-C_6) alkoxy;
 optionally, R^2 taken together with R^3 form a fused aromatic ring that is optionally
 substituted with from one to four substituents selected from halogen cyano,
 carboxy, sulfo, hydroxy, amino, mono- or di (C_1-C_6) alkylamino, (C_1-C_6) alkyl,
 (C_1-C_6) alkylthio and (C_1-C_6) alkoxy;
 the subscript p is an integer of from 1 to 3;
 W is a di-, tri- or tetra-valent linker which is acyclic, cyclic, aromatic or a
 combination thereof, having from 4 to 50 atoms selected from the group
 consisting of C, N, O, P and S and exclusive of hydrogen atoms that fill
 available valences, and further having a nitrogen atom directly connected to
 the adjacent carbonyl group;
 K is selected from the group consisting of a lone pair of electrons, H, OH, SH, NH,
 (C_1-C_8) alkyl, aryl, an amino protecting group and a hydroxy protecting
 group;
 the subscript n is 0 or 1; and when n is 1, Y is a cleavable linking group and L is a
 solid support; and when n is 0, L is a phosphoramidite or reactive functional
 group the subscript p is an integer of from 1 to 3.

53. An oligonucleotide probe having an attached 5,6-dichlorofluorescein
 dye selected from the formulae:



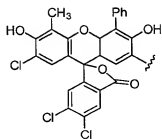
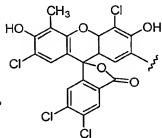
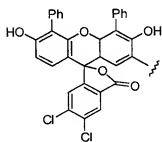
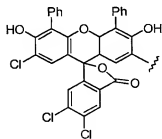
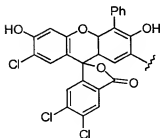
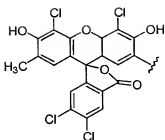
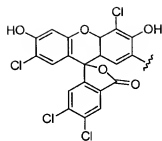
wherein,

R^x and R^y are H or independently selected protecting groups;

$R^{1'}$, R^1 , R^2 and R^4 are each independently selected from the group consisting of H, halogen, cyano, CF_3 , (C_1-C_8) alkyl, (C_1-C_8) alkylthio, (C_1-C_8) alkoxy, aryl and heteroaryl;

wherein the alkyl portions of any of $R^{1'}$ and R^1 through R^4 are optionally substituted with halogen, carboxy, sulfo, amino, mono- or dialkylamino, alkoxy, cyano, haloacetyl or hydroxy, and the alkyl portions of the substituents have from 1 to 6 carbon atoms; and the aryl portions of any of $R^{1'}$ and R^1 through R^4 are optionally substituted with from one to four substituents selected from the group consisting of halogen, cyano, carboxy, sulfo, hydroxy, amino, mono- or di (C_1-C_6) alkylamino, (C_1-C_6) alkyl, (C_1-C_6) alkylthio and (C_1-C_6) alkoxy; optionally, R^2 taken together with R^3 form a fused aromatic or heteroaromatic ring that is optionally substituted with from one to four substituents selected from halogen cyano, carboxy, sulfo, hydroxy, amino, mono- or di (C_1-C_6) alkylamino, (C_1-C_6) alkyl, (C_1-C_6) alkylthio and (C_1-C_6) alkoxy; and tautomeric forms thereof; and the wavy line indicates the point of attachment to said oligonucleotide or a linking group joining said oligonucleotide to said dye.

54. An oligonucleotide probe in accordance with claim 53, wherein said 5,6-dichlorofluorescein is selected from the group consisting of:



and

3

1 **55.** An oligonucleotide probe in accordance with claim 53, further
2 comprising an attached quencher.

1 **56.** An oligonucleotide probe in accordance with claim **53**, further
2 comprising an attached minor groove binder.

1 57. An oligonucleotide probe in accordance with claim 53, further
2 comprising an attached quencher and a minor groove binder, wherein said dye is attached at
3 the 3'-end of said oligonucleotide probe and said quencher and said minor groove binder are
4 attached at the 5'-end of said oligonucleotide probe.

1 **58.** An oligonucleotide probe in accordance with claim **53**, further
2 comprising an attached quencher and a minor groove binder, wherein said dye is attached at
3 the 5'-end of said oligonucleotide probe and said quencher and said minor groove binder are
4 attached at the 3'-end of said oligonucleotide probe.

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